

# Chromosome studies on *Vernonia flexuosa* and *V. lithospermifolia*

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## Abstract

Meiotic and mitotic chromosomes of *Vernonia flexuosa* SIMS and *V. lithospermifolia* HIERON. were analyzed in detail. *V. flexuosa* presented  $2n=4x=40$  ( $28m + 12sm$ ) and *V. lithospermifolia* showed  $2n=4x=20$  ( $10m + 2m-sm + 8sm$ ). Meiotic behaviour was regular in both species and supports an allotetraploid origin of *V. flexuosa*. The results obtained in this work could contribute to a better taxonomic identification of the two species.

## Introduction

*Vernonia flexuosa* and *V. lithospermifolia* are two closely related species distributed in Brazil, Paraguay, Argentina and Uruguay. The two species are morphologically similar, differing mainly in the head size and the leaf disposition and shape (CABRERA & KLEIN 1980). They are easily distinguished from other species of the genus by the combination of seriate-cymose inflorescence, sessile heads, yellowish-brownish pubescence on leaves and stems, and anther appendages with glands.

The two species belong to CABRERA's (1944) subsect. *Flexuosae*, or maybe better to genus *Chrysolaena* of ROBINSON (1988), which includes seven other taxa from South America, centralized geographically in southern Brazil and northern Argentina.

The cytology of this natural group is relatively well known. All the species have the basic chromosome number  $x=10$ , contrasting with most of related New World taxa, which commonly present  $x=17$  or  $x=16$  (DEMATTEIS 1997). Previous studies have reported the chromosome number of *V. lithospermifolia* (DEMATTEIS 1998) and described the karyotype of *V. flexuosa* (RUAS et al. 1991). However, to date no comparative study of the somatic and meiotic chromosomes of both entities has been made.

In this paper *V. flexuosa* and *V. lithospermifolia* are analyzed cytologically in detail with the purpose to provide data for an accurate identification of the two species.

### Materials and methods

Voucher specimens were deposited in the herbarium of the Instituto de Botánica del Nordeste (CTES). The material examined is as follows.

*V. flexuosa* SIMS: **Uruguay. Dept. Artigas.** Tomás Gomensoro. DEMATTEIS et al. 491 (CTES).

*V. lithospermifolia* HIERON.: **Argentina. Corrientes. Dept. Saladas.** Río San Lorenzo. DEMATTEIS & SOLÍS NEFFA 503 (CTES, LP).

Chromosome studies were made from root tips of germinating seeds. After a pretreatment of about 4 hours in 8-hydroxyquinoline 0,002 M, the roots were fixed in ethanol-acetic acid (3:1) and stained according to the Feulgen technique. Nomenclature used for karyotype description is that proposed by LEVAN et al. (1964). Chromosome morphology was characterized using the centromeric index ( $ci = \text{short arm} \times 100 / \text{total chromosome length}$ ). The idiograms and measures were obtained from the mean of ten metaphase plates for each species.

The fertility and diameter of the pollen grains were estimated from herbarium specimens by stain with carmin-glicerina (1:1).

### Results

Chromosome number, total chromosome length, mean chromosome length, centromeric index and pollen diameter of *V. flexuosa* and *V. lithospermifolia* are summarized in Table 1.

The somatic chromosome number of *V. flexuosa* was found to be  $2n=40$  (Fig. 1). Karyotype was composed of  $28m + 12sm$  (Fig. 3). Chromosomes ranged in length from 1,50 to 3,20  $\mu\text{m}$ . The pair 2m showed a macrosatellite in the short arm, while the pair 12m presented a microsatellite in the short arm.

In *V. lithospermifolia*  $2n=20$  chromosomes were observed (Fig. 2). The karyotype formula was composed of  $10m + 2m-sm + 8sm$  (Fig. 4). The pair 3m showed macrosatellite in the long arm and the pair 6m presented microsatellite in the short arm.

Meiotic behaviour was regular in the two species, showing always bivalents, 20II in *V. flexuosa* and 10II in *V. lithospermifolia*. Fertility of pollen grains was 95,30 % in *V. flexuosa* and 96,36 % in *V. lithospermifolia*. Results indicate that *V. lithospermifolia* is diploid on base  $x=10$ , while *V. flexuosa* in view of its meiotic behaviour would be considered allotetraploid with the same basic chromosome number.

### Discussion

Most taxonomic treatments on the *Flexuosae* group of *Vernonia* distinguish *V. flexuosa* and *V. lithospermifolia* according to the head size, number of flowers, and leaf shape (CABRERA & KLEIN 1980, JONES 1981, ROBINSON 1988). However, in several cases it is difficult to make a clear distinction between the species, due to the wide variation and apparent overlapping in these characters.

*V. flexuosa* shows a great variation in head size, ranging from (6) 7 to 12 (14) mm in length. This was first noted by HIERONYMUS (1897), who established four varieties according to this feature. *V. lithospermifolia* is particularly similar to *V. flexuosa* var. *microcephala* HIERON., with which it may be confused due to the reduced heads of the latter taxon.

Results obtained here indicate that it is possible to perform a further distinction of these species considering the cytological information. From this viewpoint, *V. flexuosa* and *V. lithospermifolia* are certainly very distinct. Besides the evident difference in ploidy level, the karyotypes differ in number of metacentrics and submetacentrics, symmetry level and total length per haploid genome.

The difference in ploidy level between the species is also evident in the diameter of the pollen grains, which is relatively constant within each species. The pollen size might be effectively used to separate herbarium material that cannot be distinguished otherwise.

Despite the differences noted above, the two species have the same number and morphology of satellites. Most members of subsect. *Flexuosae* have constantly one or two chromosome pairs with a microsatellite (DEMATTEIS 1997). *V. flexuosa* and *V. lithospermifolia* are the only two taxa of this group with both macro- and micro-satellites, which supports the close relationship between these species.

### Acknowledgements

This work was supported by grants of the SGCyT-UNNE.

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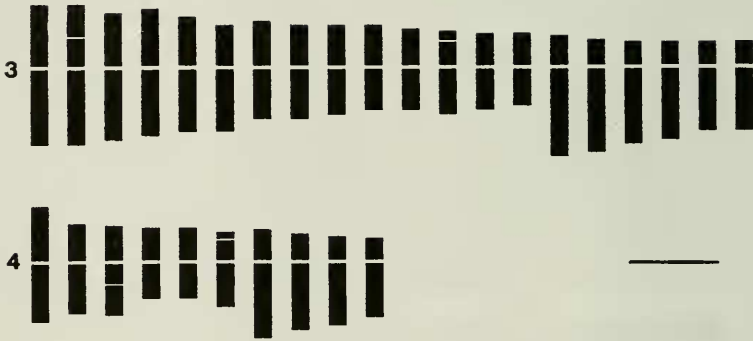
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**Table 1. Somatic chromosome number (2n), total chromosome length (TCL), mean chromosome length (ML), centromeric index (CI), and pollen diameter in  $\mu\text{m}$  ( $\phi$ ) of *V. flexuosa* and *V. lithospermifolia*.**

Species	2n	TCL	ML	CI	$\phi$ pollen
<i>V. flexuosa</i>	40	$94.60 \pm 3.70$	2.36	$41.10 \pm 0.50$	(39) 41–42 (46)
<i>V. lithospermifolia</i>	20	$40.54 \pm 2.30$	2.02	$38.88 \pm 0.23$	(31) 32–33 (36)



**Figs. 1-2.** Somatic chromosomes of *V. flexuosa*,  $2n=40$  (1), and *V. lithospermifolia*,  $2n=20$  (2). Scale = 5  $\mu\text{m}$ .



Figs. 3-4. Idiograms of *V. flexuosa*, 28m + 12sm (3) and *V. lithospermifolia*, 10m + 2m-sm + 8sm (4). Scale = 2 $\mu$ m.